



I have the touch – evidence for considerable N transfer from peas to oats by rhizodeposition

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Abstract

Aims The current study quantified the carbon (C) and nitrogen (N) transfer from peas to oats under field conditions to assess the effects of intercropping. The data obtained were compared with previously published pot and field experiments.

Methods Pea (*Pisum sativum* L. cv. Santana) and oat (*Avena sativa* L. cv. Dominik) plants were grown as intercrops for 105 days. Pea plants were labelled with a solution of 2% ^{13}C glucose (99 atom%) and 0.5% ^{15}N urea (95 atom%), using the cotton wick technique.

Results Pea rhizodeposits reached 540 kg C ha^{-1} and 17 kg N ha^{-1} . CdfR (C derived from

rhizodeposition) and NdfR corresponded to a proportion of 18.2 and 12.7%, respectively, of total pea biomass C and N. In the intercropped oat plants, only 0.6% of the total pea CdfR amount was found, but nearly 30% of the total pea NdfR amount.

Conclusions CdfR and NdfR as proportion of total pea biomass C and N, respectively, were 2.5 times higher in peas intercropped with oat plants than in sole-cropped pea plants, comparing the current results with those from previously published pot and field experiments. Future studies on intercropping should consider root formation and rhizodeposition more often.

Keywords Wick labelling method · Rhizodeposition · N Transfer · Intercropping · Soil microbial biomass

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Introduction

Organic farming systems fully rely on the N_2 fixation by legumes as a major N input source (Berry et al. 2002; Šarūnaitė et al. 2022; Watson et al. 2017). Cereals, such as oats (*Avena sativa* L.; Jannoura et al. 2013, 2014; Neugschwandter and Kaul 2014; Neumann et al. 2007) and spring barley (*Hordeum vulgare* L.; Jensen 1996; Darch et al. 2018) intercropped with grain legumes, can enhance N_2 fixation as they are more competitive for soil inorganic N than legumes. This forces nodulation and N_2 fixation, e.g., of pea (*Pisum sativum* L.) as a typical legume partner in intercropping systems in temperate arable farming (Hu et al. 2018; Jensen et al. 2020).

Intercropping of cereals with peas increases their yield stability, as this reduces weed and disease pressure (Bedoussac et al. 2015; Corre-Hellou et al. 2006; Hauggaard-Nielsen et al. 2009; Mesbah et al. 2019). An even more important benefit is the improved belowground nutrient transfer, e.g., by direct root contact, diffusion of rhizodeposits, and arbuscular mycorrhizal fungi (AMF; Garcia et al. 2016; Hupe et al. 2021). Intercropping increased pea rhizodeposits in a recent pot experiment (Hupe et al. 2021), which has effects on soil microorganisms (Oelbermann et al. 2015; Song et al. 2007; Thapa et al. 2021).

Not only easily decomposable root mucilage contributes to rhizodeposits, but also more recalcitrant remains from decaying fine roots and root fragments (Hupe et al. 2019). Consequently, the terms “rhizodeposits” referring to the compounds released and “rhizodeposition” the process of release should embrace all ions, gases, volatile organic components, exudates, lysates, and secretions released by living root cells as well as by decaying fine root fragments (Uren 2001; Wichern et al. 2008). Consequently, rhizodeposits must be considered as an important C and N source in soil as repeatedly demonstrated by N balance calculations (Mayer et al. 2003a, b; Fustec et al. 2010) and C sequestration estimates of crop rotations (Wichern et al. 2008).

In the current study, the semi-leafless summer pea variety Santana was intercropped with oat plants, accompanying the field experiment of Hupe et al. (2019). The pea plants were labelled in the field with ^{13}C and ^{15}N , using the cotton wick stem feeding technique (Chalk et al. 2014; Ingrassia et al. 2019; Russell and Fillery 1996; Wichern et al. 2007a, b).

The underlying hypotheses were: (1) Significant amounts of C and N derived from rhizodeposition are transferred from peas to oats. (2) Intercropping of pea and oat plants increases the contribution of rhizodeposits to total pea biomass C and N in comparison with other studies.

Many studies have investigated pea and oat intercropping systems without considering root formation and rhizodeposition (Gronle et al. 2015; Jannoura et al. 2013; Lauk and Lauk 2008; Šarūnaitė et al. 2022). For this reason, the current results were compared with available data on sole pea and sole oat cropping as well as pea and oat intercropping pot and field experiments to get a clearer view of their generalisable meaning.

Materials and methods

Soil

The experiment was carried out at the research station of the University of Kassel in Neu-Eichenberg, Northern Hesse, Germany (N 51°22'48.139", E 9°54'42.120", 220 m asl), which has been managed organically since 1998. In 2013, the total annual rainfall was 711 mm. During the study period (May–August 2013), rainfall was 240 mm and mean temperature was 15.3 °C (min 5.6 °C; max 25.0 °C). The silt loam was classified as a Haplic Luvisol (IUSS Working Group WRB 2022), with a soil pH- $CaCl_2$ of 6.1 and a soil texture consisting of 13% clay, 84% silt, and 3% sand (Lukas et al. 2019). Bulk density at the experimental microplots was 1.22 g cm⁻³ at 0–10 cm depth, 1.44 g cm⁻³ at 10–20 cm depth, and 1.49 g cm⁻³ at 20–30 cm depth. The soil contained 20 µg Ca-acetate lactate extractable P g⁻¹ soil, 54 µg K g⁻¹ soil, and 90 µg Mg g⁻¹ soil at 0–30 cm depth. The preceding crops were winter wheat (*Triticum aestivum* L.) in 2012 and oilseed rape (*Brassica napus* L.) in 2011. The current experiment was carried out on the same field at the same time as the study of Hupe et al. (2019) on the rhizodeposition of sole-cropped pea plants.

Experimental design and labelling

In April 2013, semi-leafless peas (*Pisum sativum* L., cv. Santana) and oats (*Avena sativa* L., cv.

Dominik) were sown manually in 20 microplots ($37.5 \text{ cm} \times 33.3 \text{ cm} = 0.125 \text{ m}^2$). The microplots were arranged in a completely randomized block design on an area of $33 \times 18 \text{ m}$. On 5 microplots, oats were cultivated as a sole crop with 18 plants at 12.5 cm distance between rows to achieve a seeding rate of 144 oat plants m^{-2} . On 5 microplots, peas were cultivated as a sole crop with 6 plants at 12.5 cm distance between rows to achieve a seeding rate of 48 pea plants m^{-2} . On the 10 intercropping microplots, 6 pea and 9 oat plants were cultivated at a 12.5 cm distance between seedlings and rows to achieve a seeding rate of 48 pea and 72 oat plants m^{-2} (Fig. 1). The soil of

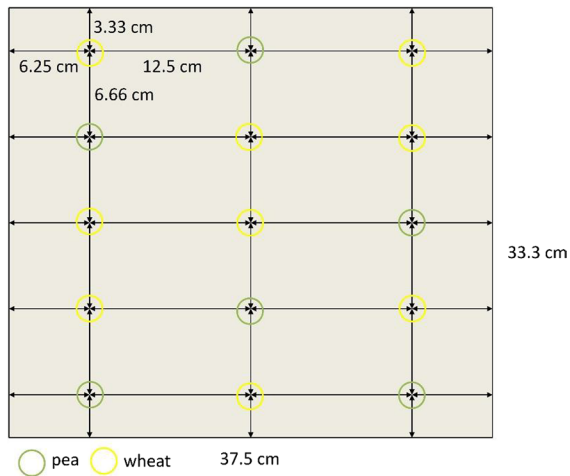


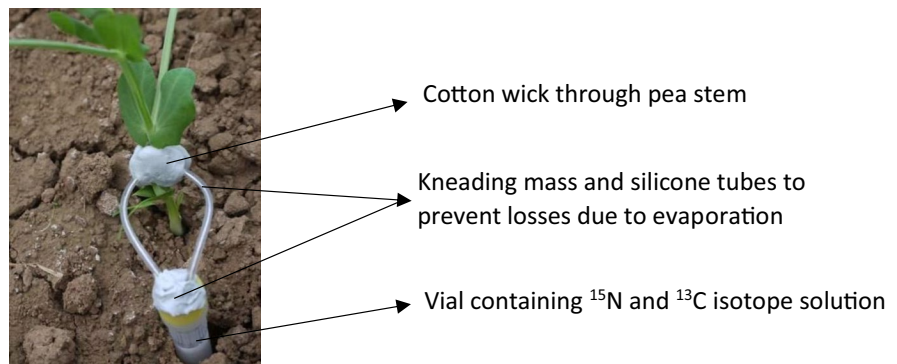
Fig. 1 Size of the micro-plots: $33.3 \times 37.5 \text{ cm}$; distance between the rows: 12.5 cm; distance within the row: 6.6 cm; pea (6 plants, green) and oat (9 plants, yellow), seeding rate: 48 peas and 72 oats per m^2

all microplots was covered with a 1 mm mesh to prevent soil contamination from falling leaves, which were collected regularly.

The cotton wick method (Russel and Fillery 1996; Wichern et al. 2007a, b) was used in 5 of the microplots, where 6 pea plants were labelled with a ^{13}C -glucose (99 atom%) and ^{15}N -urea (95 atom%) solution (Fig. 2). For this technique, the stem of each pea plant was drilled with a 0.5 mm drill, approximately 3–4 cm above the ground. Then, a small cotton wick was passed through the hole. To prevent evaporation losses and contamination of the tracer solution, the cotton wick was put through small silicon tubes. These silicon tubes (and the cotton wick) were passed through two holes in the lid of a 2 ml vial, which contained the labelling solution. A kneading mass (Teroson, Henkel, Heidelberg, Germany) was placed between stem or lid and silicon tubes to prevent evaporation losses of the labelling solution (Hupe et al. 2016). The labelling solution was produced by mixing deionized water and ^{13}C -glucose and ^{15}N -urea and then sterile filtered ($<0.2 \mu\text{m}$).

For homogeneous enrichment of ^{13}C and ^{15}N , pea plants were continuously labelled with multiple pulses of 0.5 ml labelling solution (Wichern et al. 2008), starting at 41 DAS (3 June 2013, 3 leaves unfolded) until 93 DAS (12 August 2013). Solution uptake was documented every second day. Plants usually took up most of the solution within 48 h. The vials were refilled with 0.5 ml labelling solution after complete uptake of the previous solution by the plants. All pea plants had labelling solution available until harvest. New sterile labelling systems were installed after contamination with fungi or algae, where necessary.

Fig. 2 Pea plant labelled at the third foliage leaf stage, using the cotton wick method



The manual labelling procedure was carried out with the help of a height-adjustable multicarrier (Hupe et al. 2019). This consisted of a flatbed with a hole for the head of the technician, installed on a large wooden board with four tires, having an appropriate width to cross the microplot without damaging the plants. The general recovery of the labelling solution with ^{15}N and ^{13}C was $83\% \pm 5$ standard deviations, which is like the 84% (Hupe et al. 2018) and moderately above the 68% (Hupe et al. 2021) and 64% (Hupe et al. 2019), using similar experimental conditions in previous experiments. In contrast to the accompanying field experiment of Hupe et al. (2019), no differences were detected for root, straw, and grain dry weight (DW), which is in line with Mahieu et al. (2009) and Wichern et al. (2007a, b, 2010), when using the cotton wick technique.

Plant and soil sampling

The microplots with non-labelled pea and oat intercropping and those with labelled pea and oat intercropping were harvested 106 DAS on 12 August 2013. First, the labelling system was carefully removed from the labelled pea plants. Then, the aboveground and belowground biomass of pea and oat plants of the 5 non-labelled and 5 labelled intercropping microplots were harvested completely for exact shoot and root biomass determination. The aboveground biomass of these plants was separated into grain and straw. The root biomass of pea and oat plants was excavated with a spade and manually separated according to their morphology (Jannoura et al. 2014) without the help of sophisticated technical equipment (Naumann et al. 2010). A soil sample was taken with a soil corer (a mixture of 5 subsamples per microplot) at 0–30 cm depth for measuring $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios, SOC, total N, and microbial biomass. Pea and oat plants were analyzed for isotope ratios in straw, grains, and roots. The sole pea and sole oat cropping microplots were harvested on 15 August 2013, 109 days after sowing (DAS). The aboveground, i.e., straw and grain, as well as the belowground biomass, i.e., roots excavated with a spade, of one pea plant and one oat plant was determined in each of these sole cropping microplots.

All plant parts were dried for 48 h at 60 °C for DW estimation. Subsamples of soil were sieved (< 2 mm)

to remove all visible root fragments (> 2 mm), supported by hand collecting with tweezers, and dried for 24 h at 105 °C for DW estimation. All plant and soil samples from the intercropping microplots were ground with a ball mill to measure total C and N using an elemental analyzer (Fisons, Milano, Italy), followed by measuring the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios with an isotope ratio mass spectrometer (IRMS) (Finnigan MAT, Bremen, Germany).

Microbial biomass

Microbial biomass C (MBC) and N (MBN) were estimated by fumigation extraction (Brookes et al. 1985; Vance et al. 1987), including a pre-extraction step for removing living roots (Mueller et al. 1992). Firstly, 30 g of field moist soil was horizontally shaken for 30 min with 70 ml 0.05 M K_2SO_4 at 200 rev min^{-1} and centrifuged for 10 min at 4,000 g and 6 °C. The supernatant was filtered and frozen for measuring organic C and total N as well as the isotope ratios in the pre-extracts. After pre-extraction, 15 g of the 0.05 M K_2SO_4 saturated soil was fumigated for 24 h at 25 °C with ethanol-free CHCl_3 . After CHCl_3 removal, the fumigated and 15 g of the non-fumigated pre-extracted soil were further extracted with 60 ml of 0.05 M K_2SO_4 , and the extract kept frozen until further analyses. Organic C and total N in all extracts were analysed using a C/N analyser (Multi N/C 2100S, Analytik Jena, Germany). After freeze-drying of 20 ml soil extracts, the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios were determined by EA-IRMS.

MBC was calculated as E_C / k_{EC} , where E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al. 1990). MBN was calculated as E_N / k_{EN} , where E_N = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996).

Calculations

$^{13}\text{C}_{\text{tracer}}$ derived from rhizodeposition ($= ^{13}\text{C}_{\text{tracer}} \text{dfr}$) and $^{15}\text{N}_{\text{tracer}} \text{dfr}$, i.e., in soil close to pea roots and oat plants, were calculated using the mass balance approach (Eqs. 1 and 2, see also supplementary material) as described in Hupe et al. (2016, 2019).

$$^{13}\text{C}_{\text{tracer}}\text{dfR}(\%) = ^{13}\text{C}_{\text{tracer}}\text{dfR} / ^{13}\text{C}_{\text{tracer}}\text{total} \times 100 \quad (1)$$

$$^{15}\text{N}_{\text{tracer}}\text{dfR}(\%) = ^{15}\text{N}_{\text{tracer}}\text{dfR} / ^{15}\text{N}_{\text{tracer}}\text{total} \times 100 \quad (2)$$

It was assumed that the two ratios of Eqs. (1) and (2) in soil to $^{13}\text{C}_{\text{tracer}}\text{total}$ and $^{15}\text{N}_{\text{tracer}}\text{total}$ in pea plants and rhizodeposition (in soil close to pea roots and oat plants) corresponds to the ratios of C and N in rhizodeposition to Ntotal in pea plants and rhizodeposition (in soil close to pea roots and oat plants). Consequently, $^{13}\text{C}_{\text{tracer}}\text{dfR}(\%)$ was CdfR (%) and $^{15}\text{N}_{\text{tracer}}\text{dfR}(\%)$ was NdfR (%). CdfR and NdfR in soil were separately calculated for SOC and total N, for pre-extractable C and N as well as for MBC and MBN (supplementary material). The C and N transfer from pea plants to the intercrop oat (grain, straw, and roots) were calculated as percent of CdfR ($\text{CdfR}_{\text{soil}} + \text{CdfR}_{\text{oats}}$) and percent NdfR ($\text{NdfR}_{\text{soil}} + \text{NdfR}_{\text{oats}}$) recovered in oat plants:

$$\text{CdfR}_{\text{oats}}(\%) = (\text{CdfR}_{\text{grain}} + \text{CdfR}_{\text{straw}} + \text{CdfR}_{\text{roots}}) / (\text{CdfR}_{\text{soil}} + \text{CdfR}_{\text{oats}}) \times 100 \quad (3)$$

$$\text{NdfR}_{\text{oats}}(\%) = (\text{NdfR}_{\text{grain}} + \text{NdfR}_{\text{straw}} + \text{NdfR}_{\text{roots}}) / (\text{NdfR}_{\text{soil}} + \text{NdfR}_{\text{oats}}) \times 100 \quad (4)$$

$\text{CdfR}_{\text{oats}}$ and $\text{NdfR}_{\text{oats}}$ were the amount of $^{13}\text{C}_{\text{tracer}}\text{dfR}$ and $^{15}\text{N}_{\text{tracer}}\text{dfR}$ measured in the grain, straw, and roots of oat plants derived from the labelled pea plants.

Statistics

Data are presented in tables on an oven-dry weight basis (ca. 24 h at 105 °C). Normality was tested by the Shapiro–Wilk test and equal variance by the Levene test. Data were log-transformed to normalize the

distribution if required. Differences between varieties were tested by one-way analysis of variance. Differences between plant developmental stages were tested by one-way analysis of variance, using seeding rate as covariate. Differences between pot and field experiments and CdfR and NdfR fractions were assessed using the pairwise t-test. Regression analysis was carried out between the CdfR / root-C and NdfR / root-N was evaluated after log-transformation of the data obtained by Hupe et al. (2018, 2019, 2021). Pearson correlation analysis was carried out between root-C, root-N, CdfR, and NdfR. All statistical analyses were carried out using SigmaPlot 14.0 (Systat, San José, USA).

Results

Stem feeding of pea plants with ^{13}C -glucose and ^{15}N -urea led to high ^{13}C -atom%excess and particularly ^{15}N -atom%excess values of plant tissue in the order straw > grain = roots (Table 1). Intercropped oats exhibited also small increases in ^{13}C -atom%excess, especially in the roots and stronger increases in ^{15}N -atom%excess, which varied around 0.0894 ^{15}N -atom%excess in oat plant tissues. The soil showed increased values of ^{13}C -atom%excess and ^{15}N -atom%excess only around the pea plants.

The amount of shoot-C per pea plant reached approximately 86% of that per intercropped oat plant (Table 2), whereas the amount of shoot-N was identical in both crops. In contrast, root-C per plant was similar for both crops, whereas root-N was 30% higher per pea plant than per oat plant. Consequently, the shoot-C/N ratio of oat plants was only 14% higher than that of pea plants, whereas the root-C/N ratio of oat plants was 40% higher. The shoot-C / root-C and shoot-N / root-N ratios were 27 and 20% lower, respectively, in the pea than in

Table 1 ^{13}C -atom%excess and ^{15}N -atom%excess in grain, straw, and roots of pea and intercrop oat as well as in soil near pea plants

	^{13}C -atom%excess ($\pm$ SEM)		^{15}N -atom%excess ($\pm$ SEM)	
	Pea	Intercrop oat	Pea	Intercrop oat
Grain	0.2244 $\pm$ 0.0653	0.0003 $\pm$ 0.0001	3.5442 $\pm$ 0.9151	0.0893 $\pm$ 0.0262
Straw	0.5761 $\pm$ 0.0891	0.0001 $\pm$ 0.0001	5.7265 $\pm$ 0.8137	0.0900 $\pm$ 0.0236
Roots	0.2686 $\pm$ 0.0286	0.0017 $\pm$ 0.0002	2.8102 $\pm$ 0.4634	0.1158 $\pm$ 0.0205
Soil	0.0012 $\pm$ 0.0002		0.0058 $\pm$ 0.0012	

SEM = standard error of mean

Table 2 C and N in shoots (grain + straw), roots, and derived from rhizodeposits (dfR) as well as the respective shoot-to-root and RD-to-root ratios in pea and intercrop oat plants

	Pea	Intercrop oat
C ($\pm$ SEM)		
Shoot-C (mg plant ⁻¹)	4557 $\pm$ 701	5290 $\pm$ 454
Root-C (mg plant ⁻¹)	211 $\pm$ 38	230 $\pm$ 54
Shoot-C / root C	22 $\pm$ 1	28 $\pm$ 6
CdfR (mg plant ⁻¹)	1188 $\pm$ 505	
CdfR / root C	5.7 $\pm$ 2.1	
N ($\pm$ SEM)		
Shoot-N (mg plant ⁻¹)	207 $\pm$ 30	211 $\pm$ 43
Root-N (mg plant ⁻¹)	7.9 $\pm$ 1.6	6.1 $\pm$ 0.6
Shoot-N / root N	30 $\pm$ 5	36 $\pm$ 9
NdfR (mg plant ⁻¹)	36 $\pm$ 18	
NdfR / root N	4.6 $\pm$ 1.9	
C/N ($\pm$ SEM)		
Shoot-C/N	22 $\pm$ 1	27 $\pm$ 3
Root-C/N	27 $\pm$ 3	36 $\pm$ 6
C/N-dfR	33 $\pm$ 6	

SEM = standard error of mean

the oat plants. The amounts of CdfR and NdfR per pea plant can be recalculated into amounts in kg ha⁻¹ by multiplication by the seeding rate of 48 pea plants m⁻², which results in rhizodeposits of 540 kg C ha⁻¹ and 17 kg N ha⁻¹ and a C/N ratio of 33 in the rhizodeposits.

MBC-dfR was below 3% but exceeded that of MBN-dfR (Table 3), leading to a markedly higher MB-¹³C/¹⁵N ratio in comparison with MB-CN ratio of 8.5. Extractable CdfR contributed 1.3% to extractable organic C. In contrast, extractable NdfR contributed 2.7% to extractable N, i.e., MBC and

extractable C contributed less to CdfR than MBN and extractable N to NdfR. CdfR and NdfR corresponded to a proportion of 18.2 and 12.7%, respectively, of total pea biomass C and N (Table 4).

In the intercropped oat, pea CdfR and particularly pea NdfR were mainly found in the oat grain, followed by the oat straw, whereas a ten-fold smaller percentage was found in the small amounts of oat roots. The transfer of pea rhizodeposits to oat plants was 5.05 kg CdfR ha⁻¹ (1188 mg CdfR pea plant⁻¹ $\times$ 0.59% CdfR_{oats} / 100 $\times$ 72 oat plants = 505 mg CdfR m⁻²) and 7.72 kg NdfR ha⁻¹ (36 mg NdfR pea plant⁻¹ $\times$ 29.8% NdfR_{oats} / 100 $\times$ 72 oat plants = 772 mg NdfR m⁻²).

Discussion

C and N transfer from pea to oat

Small, but significant percentages of pea rhizodeposits were transferred by direct root contact, AMF, or diffusion to oats (Hupe et al. 2019). If all the 5.05 kg CdfR ha⁻¹ in intercropped oats was transferred by AMF with a C/N ratio of 33 (Table 2), 0.15 kg NdfR ha⁻¹ or approximately 0.9% of NdfR was transferred by the AMF pathway to oats.

This percentage is close to the low 0.7% of NdfR transferred by AMF from the winter pea Frisson to triticale in a pot experiment (Hupe et al. 2021), considering the uncertainties of the current assumptions and the large variation between the replicates. In the study of Hupe et al. (2021), 2.7% of pea CdfR and 6.7% of pea NdfR were on average transferred to

Table 3 Mean contents of microbial biomass C (MBC), and microbial biomass N (MBN), soil organic C (SOC), total N, 0.05 M K₂SO₄ extractable organic C, 0.05 M K₂SO₄ extract-

able total N, the contribution of respective fractions derived from rhizodeposition (dfR) as well as the ratios MB-C/N and MB-¹³C/¹⁵N

	C ($\pm$ SEM)		N ($\pm$ SEM)
MBC (μ g g ⁻¹ soil)	179.1 $\pm$ 20.6	MBN (μ g g ⁻¹ soil)	21.2 $\pm$ 2.3
MBC-dfR (%)	2.9 $\pm$ 1.5	MBN-dfR (%)	1.9 $\pm$ 1.0
MB-C/N	8.5 $\pm$ 0.6	MB- ¹³ C/ ¹⁵ N	19.1 $\pm$ 1.4
SOC (mg g ⁻¹ soil)	10.9 $\pm$ 0.3	Total N (mg g ⁻¹ soil)	1.24 $\pm$ 0.1
SOC-dfR (%)	1.2 $\pm$ 0.5	Total N-dfR (%)	0.22 $\pm$ 0.11
Extractable C (μ g g ⁻¹ soil)	8.0 $\pm$ 0.2	Extractable N (μ g g ⁻¹ soil)	2.3 $\pm$ 0.2
Extractable C-dfR (%)	1.3 $\pm$ 0.4	Extractable N-dfR (%)	2.7 $\pm$ 1.1

SEM = standard error of mean

Table 4 CdfR and NdfR as proportion of total pea biomass C and N, respectively; contribution of MBC, SOC, extractable organic C, and soil to CdfR; contribution of MBN, total N,

extractable total N, and soil to NdfR; contribution of CdfR and NdfR to C and N in grain, straw, and roots of intercrop oat

	C ($\pm$ SEM)		N ($\pm$ SEM)
CdfR (% total pea biomass C)	18.2 $\pm$ 5.4	NdfR (% total pea biomass N)	12.7 $\pm$ 4.9
% of pea CdfR in:		% of pea NdfR in:	
MBC	4.9 $\pm$ 1.3	MBN	12.0 $\pm$ 3.0
Extractable C	0.11 $\pm$ 0.03	Extractable N	6.2 $\pm$ 0.7
SOC	99.4 $\pm$ 0.3	Total N	70.2 $\pm$ 5.1
% of pea CdfR in intercrop oat:		% of pea NdfR in intercrop oat:	
Grain	0.27 $\pm$ 0.12	Grain	17.3 $\pm$ 4.0
Straw	0.16 $\pm$ 0.16	Straw	11.2 $\pm$ 1.8
Roots	0.17 $\pm$ 0.02	Roots	1.3 $\pm$ 0.3
Total	0.59 $\pm$ 0.27	Total	29.8 $\pm$ 5.1

SEM = standard error of mean

triticale, which is markedly more CdfR and considerably less NdfR than in the current study. Approximately 90% of this NdfR was transferred by direct root contact, 10% by AMF and nothing by diffusion from the pea Frisson to triticale. It was surprising that similar percentages were transferred from the non-mycorrhizal Frisson isoline mutant P2 to triticale in this experiment (Hupe et al. 2021). However, triticale-colonizing AMF were most likely able to transfer organic and inorganic ^{15}N and ^{13}C components to their cereal host, taken up in the rhizosphere of mutant P2 without being directly connected to the pea plants.

AMF are most likely not only responsible for the direct transfer from pea to a cereal but also for the strong positive effects of direct root contact. This might explain the much higher AMF-related transfer rates reported in the literature (He et al. 2009). For example, AMF contributed up to 15% of the total N transfer from pea to barley (*Hordeum vulgare*) in a controlled laboratory environment (Johansen and Jensen 1996), using a split-root labelling technique. AMF supplied even up to 24% of the total N transfer from faba bean (*Vicia faba* L.) to intercropped durum wheat (*Triticum durum* Desf.), using the ^{15}N isotope dilution method (Wahbi et al. 2016).

Root development and rhizodeposition

In the available studies (Table 5), the shoot-to-root ratio varied around medians of 6.0, 17, and 8.6 in sole

oat, sole pea, and pea and oat intercropping experiments (Table 5). The shoot-to-root ratio of oat plants did not differ significantly between flowering and dry maturity, in contrast to pea plants, which exhibited markedly lower shoot-to-root ratios at flowering (10.4) than at dry maturity (29). In the intercropped pea and oat plants, the shoot-to-root ratio of the current study markedly exceeded that of the other four studies, which varied around 7.8 (Freund et al. 2021; Jannoura et al. 2014; Kwabiah et al. 2005). An important reason might be the higher water availability in the current field experiment. For example, at dry maturity, the shoot-to-root ratio of Frisson decreased from 43 for plants grown in pots at 80% water holding capacity to 15 for those grown at 40% (Kleikamp and Joergensen 2006). Another reason for a high shoot-to-root ratio of mature pea plants might be a strong root decay (Hupe et al. 2019) caused by intense fungal colonization during maturation (Jannoura et al. 2014).

The root DW yield of intercropped pea and oat mix obtained in the current study was above that observed in the two field studies (Jannoura et al. 2014; Kwabiah et al. 2005) but markedly below that of one pot experiment (Freund et al. 2021) previously published. In contrast to the shoot-to-root ratio, the root DW yield was always affected by the plant development stage (Table 5), but in the case of oats different to the expectations stated by Kwabiah et al. (2005). In oat cropping studies, the geometric mean root yield at flowering (860 kg DW ha⁻¹) was significantly lower ($P < 0.03$) than that at dry maturity (2070 kg DW

Table 5 Cultivation, seeding rate, plot size or pot surface, harvest stage, sampling depth, shoot-to-root DW ratio, and total yield in sole oat and sole pea cropping experiments as well as in pea and oat intercropping (intercr.) experiments

Reference	Cultivation	Seeding rate (m ⁻²)		Plot (m ²)	Pot (cm ²)	Harvest	Depth (cm)	Shoot-to-root DW			Root DW (kg ha ⁻¹)		
		Oat	Pea					Oat	Pea	Intercr.	Oat	Pea	Intercr.
Bolinder et al. (1997)	Sole	400		3		Dry maturity	30	2.5			3390		
Kwabiah et al. (2005)	Sole	170	150	7.2		Dry maturity ^a	30	8.6	11		730	370	
	Intercropped	85	150	7.2		Dry maturity ^a	30			8.6			540
	Intercropped	170	150	7.2		Dry maturity ^a	30			9.0			770
	Intercropped	170	150	7.2		Dry maturity ^a	30						
Pietola et al. (2005)	Sole	500		25		Dry maturity	60	4.4			2580		
Kleikamp et al. (2006) ^b	Sole		40		870	Dry maturity	20		43			360	
Jannoura et al. (2012) ^c	Sole		89		225	Flowering	20		4.5			980	
Jannoura et al. (2014) ^c	Sole	300	80	27		Flowering	30	4.5	21		1270	120	
	Intercropped	60	80	27		Flowering	30	8.5	14	7.9	320	130	450
Hupe et al. (2018)	Sole		89		225	Flowering	20		14			90	
	Sole		89		225	Dry maturity	20		32			140	
Hupe et al. (2019)	Sole		64	0.19		Flowering	90		4.9			570	
	Sole		64	0.19		Dry maturity	90		28			100	
Freund et al. (2021)	Sole	400	150		490	Flowering	25	3.1	3.4		1850	2520	
	Intercropped	163	102		490	Flowering	25			3.6			2280
Current study ^d	Sole	144	48	0.125		Dry maturity	30	7.5	20		2170	370	
	Intercropped	72	48	0.125		Dry maturity	30	7.8	22	15	960	230	1190

Shoot: grain DW + straw DW; ^a roots were sampled at flowering; ^b treatment W1; ^c non-amended control treatment with winter-pea Frisson; ^d plots without stem-feeding of pea; grey: not applicable.

ha⁻¹), integrating seeding rate as significant covariate ($P < 0.01$) into the ANOVA. In contrast, in pea cropping studies, the geometric mean root yield at flowering (360 kg DW ha⁻¹) exceeded that at dry maturity (210 kg DW ha⁻¹) without effect of the seeding rate. However, this difference between the means was not significant due to the large variation between the studies, caused by the distinct cropping conditions and the low number of studies. This also explains the absence of significant difference between field and pot studies, where the shoot-to-root ratio and the root yield of oat plants showed a stronger response to the reduction in rooting space than the root yield of pea plants.

The ratios of CdfR / root-C and NdfR / root-N obtained from the current study and previously published experiments (Hupe et al. 2018, 2019, 2021) varied around medians of 4.6 and 4.0, respectively. The two ratios showed a significant positive correlation with $r = 0.84$ (Fig. 3), although different varieties were used, leafy winter peas Frisson (Hupe et al. 2018, 2019, 2021), its non-mycorrhizal isoline mutant P2 (Hupe et al. 2021), and the semi-leafless summer pea Santana (current study). The ratios of CdfR / root-C and NdfR / root-N did not differ

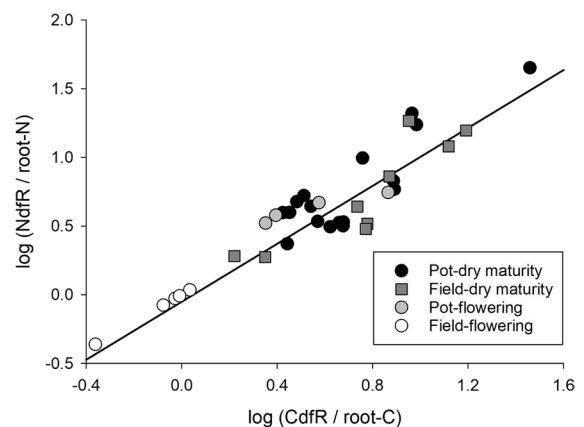


Fig. 3 Relationship of the ratios NdfR / root-N and CdfR / root-C obtained in the current field study, in two pot experiments (Hupe et al. 2018, 2021), and another field study (Hupe et al. 2019); $y = -0.051 + 1.055x$, $r = 0.84$, $P < 0.01$, $n = 35$; dfr = derived from rhizodeposition

between the three varieties (Tukey-test, $P > 0.05$). This suggests that NdfR could be assessed by information on CdfR and root-C, as also root-N and root-C were closely correlated ($r = 0.97$, $n = 9$, $P < 0.01$). However, CdfR and NdfR were not interrelated and

also not correlated with root-C or root-N. This is the reason for the attractive suggestion of solely using root DW for the estimation of CdfR under field conditions (Pausch et al. 2013). Although the ratios of CdfR / root-C and NdfR / root-N did not significantly differ between field and pot experiments and also not between flowering and dry maturity, the possibility of such differences should be considered in future experiments, as both ratios were lower in field than in pot studies and lower at flowering than at dry maturity.

Effect of intercropping on rhizodeposition

Under sole pea cropping, the proportion of CdfR (% total pea biomass C) and NdfR (% total pea biomass N) varied both around a mean of 6.8% at dry maturity. This means no differences occurred between CdfR and NdfR (Fig. 4), although it tends to be higher in the field than in a pot experiment (Hupe et al. 2018, 2019). This slight difference between pot and field experiments diminishes under pea intercropping with triticale and oat, with a significant increase ($P < 0.002$, two-tailed t-test) in the mean proportion of CdfR (% total pea biomass C) and NdfR (% total pea biomass N) to generally 16.1% at dry maturity (Hupe et al. 2019, current study). The increase in

pea rhizodeposits by intercropping with oats suggests that even a small increase in biodiversity due to more crops may have general positive effects on the soil microbiome beyond microbial biomass (Chen et al. 2019, 2018; Prommer et al. 2020). Completely unknown are the effects of suppressing the N_2 fixation by stem-feeding with urea on the N transfer rates by rhizodeposition.

However, the higher CdfR and NdfR as proportion of total pea biomass C and N, respectively, in pea intercropping studies (Hupe et al. 2021; current study) were not reflected by higher percentages of CdfR and NdfR transferred to MBC-dfR and MBN-dfR (Fig. 4) in comparison with sole pea cropping studies (Hupe et al. 2018, 2019). Similarly to CdfR and NdfR as the proportion of total pea biomass C and N (Fig. 4), no significant differences occurred in the contribution of MBC-dfR to CdfR and MBN-dfR to NdfR. In contrast, significantly ($P < 0.005$, two tailed t-test) more CdfR and NdfR (mean 5.1%) was transferred to MBC-dfR in pot experiments compared with field experiments (mean 2.0%). The main reason is the higher root density in pots, followed by a higher root turnover, i.e., formation and microbial-assisted decay of roots (Hupe et al. 2021).

Conclusions

Total pea CdfR and NdfR reached 540 kg C ha^{-1} and 17 kg N ha^{-1} . In the intercropped oat, approximately only 0.6% of this total pea CdfR amount was found, but nearly 30% of the total pea NdfR amount, which confirms our first hypothesis only for N. Rhizodeposition-N might presumably be assessed by information on rhizodeposition-C and root C. In accordance with our second hypothesis, CdfR and NdfR as proportion of total pea biomass C and N, respectively, were 2.5 times higher in peas intercropped with oat plants than in sole-cropped pea plants (16.1% versus 6.8%, respectively), comparing the current results with those from previously published pot and field experiments. The strong increase in rhizodeposits by intercropping suggests that even a small increase in crop diversity has a strong positive impact on the C transfer by plants into soil. Future studies on intercropping should more often consider root formation and rhizodeposition, using a larger number of replicates.

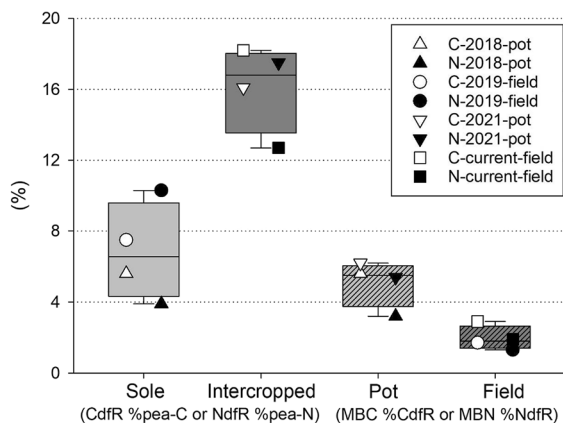


Fig. 4 CdfR and NdfR as proportion of pea biomass C (% pea-C) and biomass N (% pea-N), respectively, observed in two sole pea (Hupe et al. 2018, 2019) and two intercropped pea experiments (Hupe et al. 2021; current study) as well as the contribution of MBC and MBN in %CdfR and %NdfR, respectively, obtained in two pot (Hupe et al. 2018, 2021) and two field experiments (Hupe et al. 2019, current study); dfR = derived from rhizodeposition; MB = microbial biomass

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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